REMARKS

Previously, claims 1, 2, 5, 6, 8, 10, 13, 15, and 16 were under consideration. Claims 4, 7, 9, and 18 were withdrawn from consideration by the Patent Office for not encompassing the elected species. In the instant amendment, claims 2 and 13 are canceled, without prejudice to Applicants' right to pursue the subject matter of the canceled claims in subsequent applications. Claims 1, 5, 8, 10, 15, and 16 are amended, without prejudice to Applicants' right to pursue in subsequent applications subject matter removed by amendment to claim 1. After entry of the amendment, claims 1, 5, 6, 8, 10, 15, and 16 will be pending and under consideration.

I. AMENDMENT TO THE CLAIMS

Claims 2 and 13 have been canceled, without prejudice.

Claim 1 has been amended. The amendment to claim 1 is supported by specification, for example, page 2, lines 24-30, and page 4, lines 24-34 (line numbering from substitute specification mailed July 3, 2002), and the claims as originally filed.

Claims 5, 8, 10, 15, and 16 have each been amended to independent form incorporating the limitations of base claim 1.

As the above amendments to claims are fully supported by the specification and claims as originally filed, entry thereof is respectfully requested. No new matter has been added.

II. DRAWINGS

Enclosed are two sheets of replacement drawings labeled "3a" and "3b," which Applicants respectfully submit are in conformity with 37C.F.R. § 1.84(h). Applicants respectfully request the Patent Office to approve the newly submitted drawings.

III. WITHDRAWN CLAIMS

In the Office Action dated May 20, 2003, the Patent Office withdrew claims 4, 7, 9, and 18 from consideration, by stating that these claims do not encompass the elected species. Previously, Applicants responded to the Restriction and Election of Species Requirement (Office Action dated November 24, 2000) by filing an Amendment and Response To Restriction Requirement on January 16, 2001, to elect Group I (claims 1, 2, 4-10, 13, 15, and 16), and by filing a Response To Election Requirement on March 13, 2001, in which the species elected included a human serum albumin ("HSA") carrier, a sulfonamide linkage

between carrier and fluorescent moiety, one HSA per conjugate and an approximate excitation wavelength of 650 nm. Claims 4, 7, 9, and 18 were not withdrawn by the Patent Office in the Office Action dated May 31, 2001, or after the CPA request, in Office Actions dated January 4, 2002, and September 12, 2002. (Claim 18 was added by Applicants' Amendment and Response mailed July 3, 2002). Applicants respectfully submit that claims 7 and 18 are directed towards a subgenera of conjugates encompassed in claim 1.

Applicants respectfully request reconsideration of the withdrawal of the claims 4, 7, 9, and 18. Although the elected species is not encompassed by claims 4, 7, 9, and 18, Applicants respectfully submit that it would not seriously burden the Patent Office to consider the elected species in conjunction with conjugates comprised of, for example, a plurality of fluorescent moieties (as recited in claim 9, for instance), or a plurality of carriers (as recited in claim 4, for instance).

IV. CLAIM OBJECTIONS

The Patent Office objects to claims 5, 6, 8, 10, 15, and 16, allegedly for depending from rejected claims. Claims 5, 8, 10, 15, and 16 have each been amended to independent form. Claim 6 depends from claim 15. Hence, Applicants respectfully request that the objection to claims 5, 6, 8, 10, 15 and 16 be withdrawn.

V. REJECTION OF CLAIMS 1, 2, AND 13 UNDER 35 U.S.C. § 102(b)

Claims 1, 2, and 13 stand rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Carlsson (U.S. Pat. No. 4,231,999). The Patent Office alleges that the claims are anticipated because Carlsson teaches fluorescein bonded to albumin via an amide linkage. The rejections of claims 2 and 13 are moot in view of the cancellation of claims 2 and 13. Applicants respectfully traverse the rejection of claim 1.

For a prior art reference to anticipate in terms of 35 U.S.C. § 102, every element of the claimed invention must be identically shown in a single reference. *See In re Bond,* 15 U.S.P.Q.2d 1566, 1567 (Fed. Cir. 1990). In this instance, amended claim 1 recites, in relevant part, a "conjugate suitable for use *in vivo* . . ." that is not anticipated by Carlsson since Carlsson does not teach a conjugate suitable for use *in vivo*.

Carlsson discloses a method for preparing a fluorescein/GSH/ linker/albumin conjugate in which fluorescein is covalently linked to glutathione ("GSH") through a thiourea bond, which in turn is covalently linked via a disulfide bond to -S-CH_2-CH_2-CO- ("linker"), which in turn is linked to albumin via an amide linkage (col. 15, line 41, to col. 16, line15).

The fluorescein/GSH/ linker/albumin conjugate disclosed by Carlsson, however, is not suitable for use *in vivo*. For instance, the covalent attachment of the fluorescein to albumin includes a disulfide linkage that would be reduced in an intracellular environment to result in separation of fluorescein from albumin. *See, e.g.*, page 707, col. 2, third paragraph, of Lodish *et al.*, *Molecular Cell Biology* (4^{th} edition, 2000) (**Exhibit 1**). In contrast, as stated in the specification,"[c]onjugates according to the invention distinguish themselves by a prolonged half life in the organism" (page 4, lines 23-24), and more specifically, the conjugate recited in amended claim 1, is "suitable for use *in vivo*." For these reasons, Carlsson does not teach each and every limitation of claim 1. Hence, Applicants respectfully request that the rejection of claim 1 under 35 U.S.C. § 102(b) be withdrawn.

VI. REJECTION OF CLAIM 1 UNDER 35 U.S.C. § 103

Claim 1 stands rejected under 35 U.S.C. § 103, allegedly for obviousness in view of Tryggvason (U.S. Pat. No. 4,677,058). Applicants traverse the rejection of claim 1.

The Patent Office bears the initial burden of establishing a *prima facie* case of obviousness under 35 U.S.C. § 103. *In re Fine* , 5 U.S.P.Q.2d 1596, 1598 (Fed. Cir. 1988); MPEP § 2142. The legal standard of *prima facie* obviousness requires that three criteria be met: (1) the prior art, either alone or combination, must teach or suggest each and every limitation; (2) a suggestion or motivation in the cited references or in the art to modify or combine the cited references; and (3) the cited references must provide a reasonable expectation of successfully achieving the claimed invention. *See In re Vaeck*, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991); *In re Rouffet*, 47 U.S.P.Q.2d 1453, 1456 (Fed. Cir. 1998); *In re* Wilson, 165 U.S.P.Q. 494, 496 (CCPA 1970). Applicants respectfully submit that *prima facie* obviousness has not been established since these criteria are not met.

Amended claim 1 recites, in pertinent part, a conjugate comprising a carrier "wherein said carrier is human serum albumin." Applicants respectfully submit that amended claim 1 is not obvious in view of Tryggvason.

Specifically, Tryggvason does not teach or suggest a conjugate wherein the carrier protein is human serum albumin. Tryggvason discloses a fluorescein-conjugated goat anti-rabbit serum used to detect rabbit anti-mouse type IV collagenase serum for a study of the localization of malignant breast cancer tissues (*e.g.*, col. 6, lines 19-34). Hence, the cited reference does not teach or suggest each and every limitation of claim 1. Moreover, Applicants respectfully submit that the Patent Office has neither supplied a suggestion or

motivation in Tryggvason, or in the art, to modify Tryggvason to arrive at the conjugate recited in claim 1.

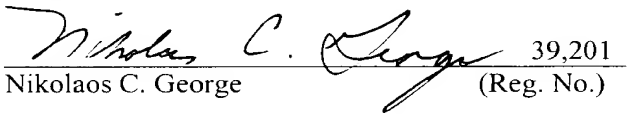
For these reasons, Applicants respectfully submit that *prima facie* obviousness has not been established. Accordingly, Applicants respectfully request that the rejection of claim 1 under 35 U.S.C. § 103 be withdrawn.

CONCLUSION

In light of the above amendments and remarks, Applicants respectfully request that the Patent Office reconsider this application with a view towards allowance. The Patent Office is invited to call the undersigned attorney if a telephone call could help resolve any remaining items.

Respectfully submitted,

Date: October 31, 2003



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Nikolaos C. George (Reg. No.)

PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

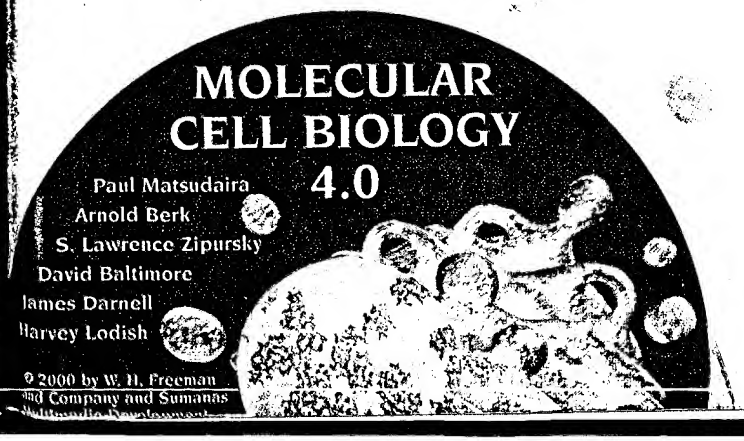
EXHIBIT 1

(Photocopies comprising three (3) sheets from Lodish *et al.*, *Molecular Cell Biology* (4th edition, 2000))

FOURTH EDITION

MOLECULAR CELL BIOLOGY

Harvey Lodish
Arnold Berk
S. Lawrence Zipursky
Paul Matsudaira
David Baltimore
James Darnell



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SUMMARY Insertion of Membrane Proteins into the ER Membrane

- Topogenic sequences direct membrane proteins synthesized on the rough ER to assume their appropriate orientation in the ER membrane. This orientation is retained during transport of a membrane protein to its final destination.
- Topogenic sequences include N-terminal cleaved signal sequences; stop-transfer membrane-anchor sequences; internal uncleaved signal-anchor sequences; and GPI-attachment sequences.
- Many proteins have several membrane-spanning α helices. Each α-helical segment in such multipass proteins functions as an internal uncleaved signal-anchor sequence or a stop-transfer membrane-anchor sequence depending on its location in the polypeptide chain (see Figure 17-24).

17.6 Post-Translational Modifications and Quality Control in the Rough ER

Newly synthesized polypeptides in the membrane and lumen of the ER undergo five principal modifications before they reach their final destinations:

1. Formation of disulfide bonds
2. Proper folding
3. Addition and processing of carbohydrates
4. Specific proteolytic cleavages
5. Assembly into multimeric proteins

The first two and the last of these modifications, which take place exclusively in the rough ER, are discussed in this section. Although addition of some carbohydrates and some proteolytic cleavages also occur in this organelle, many such modifications take place in the Golgi complex or forming secretory vesicles; we discuss these in later sections.

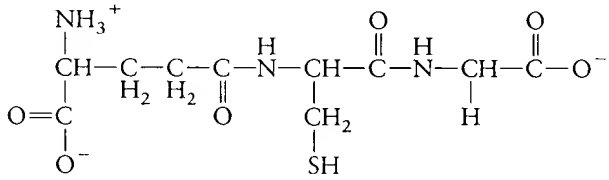
Only properly folded and assembled proteins are transported from the rough ER to the Golgi complex and ultimately to the cell surface or other final destination. Unfolded, misfolded, or partly folded and assembled proteins are selectively retained in the rough ER, or are retrieved from the *cis*-Golgi network and returned to the ER. Misfolded proteins and unassembled subunits of multimeric proteins often move from the ER lumen back through the translocon into the cytosol where they are degraded in proteasomes. We consider several examples of such "quality control" in the second half of this section.

Disulfide Bonds Are Formed and Rearranged in the ER Lumen

In Chapter 3 we learned that both intramolecular and intermolecular **disulfide bonds** (–S–S–) help stabilize the tertiary and quaternary structure of many proteins. These covalent bonds form by the oxidative linkage of **sulfhydryl groups** (–SH), also known as *thiol* groups, on two cysteine residues in the same or different polypeptide chains (see page 53). This reaction can proceed spontaneously only when sufficient oxidant is present. In eukaryotic cells, disulfide bonds are formed in the lumen of the rough ER but not in the cytosol. Thus disulfide bonds are found only in secretory proteins and in the exoplasmic domains of membrane proteins synthesized on the rough ER; soluble cytosolic proteins, synthesized on free ribosomes, lack disulfide bonds and depend on other interactions to stabilize their structures.

Localization of disulfide-bond formation in cells to the ER lumen indicates that this organelle has a favorable redox environment for oxidation of –SH groups, whereas the cytosol does not. The intracellular oxidant required for this reaction has not been identified. However, a mutation in one ER membrane protein renders yeast cells unable to generate disulfide bonds, suggesting that this protein may participate in oxidation of –SH groups in the ER lumen.

The tripeptide *glutathione*, often abbreviated G,



is the major thiol-containing molecule in eukaryotic cells, and serves to prevent the formation of disulfide bonds in the cytosol. Glutathione shuttles between the reduced form (GSH) and the oxidized form, a disulfide-linked dimer (GSSG). The GSH:GSSG ratio is over 50:1 in the cytosol; oxidized GSSG in the cytosol is reduced by the enzyme glutathione reductase, using electrons from the potent reducing agent NADPH (see Figure 16-4):

$$NADPH + H^+ + GSSG \longrightarrow NADP^+ + 2\ GSH$$

Thus cytosolic proteins in bacterial and eukaryotic cells do not utilize the disulfide bond as a stabilizing force because the high GSH:GSSG ratio would drive the system in the direction of Cys–SH and away from Cys–S–S–Cys.

In proteins that contain more than one disulfide bond, the proper pairing of cysteine residues is essential for normal structure and activity. Disulfide bonds sometimes are formed sequentially while a polypeptide is still growing on the ribosome. For instance, during synthesis of the immunoglobulin (Ig) light chain, which contains two disulfide bonds, the first and second cysteines closest to the N-terminus form a disulfide bond before the third cysteine has even been added